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HISTOLOGICAL STUDIES ON THE GONADS OF THE CATFISH *CLARIAS MAGUR* (HAMILTON, 1822) FROM UTTAR PRADESH OF INDIA WITH A NOTE ON ITS SYSTEMATIC ACCOUNT

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ABSTRACT

Histological studies on the gonads of Clariasmagur were undertaken to describe the maturity stages and understand the mode of gonad development. Based on the histological studies, six stages of oocytes development were identified. The seasonal variations in the average weight of the ovary, along with the ova diameter and Gonadosomatic Index suggests that the spawning period of this fish is from last part of June to the first part of September and the fish attains its maturity for spawning after the commencement of good rain. A systematic account of Clariasmagur is provided.

KEYWORDS: Clariasmagur, Ovary Weight, Body Length, Gonads & Systematic Account

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INTRODUCTION

Clariasmagur is a freshwater catfish of the family Clariidae and native to India, Bangladesh, Bhutan, and Nepal. They are commonly found in freshwater and brackish water and thrives well in rivers and tanks including cloudy and low-oxygen waters. Due to overexploitation as a food fish, threats to breeding grounds due to wetland conversion and pesticides in paddy fields, and from the introduction of the Thai magur, it has undergone a significant decline in population throughout its range (Vishwanath, 2010). It is commercially valuable food fish in the state of Uttar Pradesh. For conservation and sustainable production of this commercially important food fish, detail studies on the biology of the fish are required. Many workers have studied fish histology (Yoakim, 1971; Hoar and Randall, 1969; Patt and Patt, 1969; Ali and Anctil, 1976; Roberts, 1978). However, there is no detail information on the histology of Clariasmagur, especially on its reproductive biology. Therefore, gonadal development study of Clariasmagur was taken up to provide inputs for the management of the fishery of the species.

MATERIALS AND METHODS

Fishes of the catfish genus *Clarias* were collected from Ganga canal around the border of Unnao & Raibareli Districts, located on the side of the Ganga River, Uttar Pradesh during 2009-11 and identified following Ng & Kottelat (2008). Live, matured wild specimen of Clariasmagur were selected for histological studies. Collections were made during mid may & late June as pre-spawning ones, late November & mid-January, & mid-August to represent spawning season. Live specimens have immersed in 10% neutral formalin & required cuts

have been made to allow quick & deep penetration of fixative.

Gonadal Activity

The weight of the fish and that of the ovary were recorded for calculating GSI (Gonado Somatic Index) using the formula provided by Pickford (1953). This index provided an assessment of gonadal activity. This index was applied in both cases but has been recorded well in females as males showed a similar and corresponding trend except that their gonadal weights were almost less than 1/10th of those of females. Selected sections of the middle region of the ovary, prepared by routine paraffin block cutting were used for histological studies. The ova diameters were measured in 300 samples of oocytes equally asserted from the anterior, middle and posterior portions of the ovary during May, June, August & November, December using an ocular micrometer. Fresh ovaries were dissected out and cut into pieces and different samples fixed in 10% neutral formalin for histological procedures. Paraffin sections of 5-7 microns thickness were cut and stained in Eosin hematoxylin& the histoarchitecture of above said tissues were studies & recorded.

RESULTS & DISCUSSIONS

Systematic Accounts of *Clariasmagur*

A total of 32 specimens of Clariasmagur were examined and identified. The systematic accounts of the species is provided below:

Phylum - Chordata

Class - Actinopterygii

Order -Siluriformes

Family - Clariidae

Genus - *Clarias*

Clariasmagur (Hamilton, 1822)

Figure 1: Dorsal View of Clariasmagur, Hamilton, 1822

Macropteronotusmagur: Hamilton, 1822:145, 374 [An account of the fishes found in the river Ganges; Type locality: Ganges River, India.

Clariasmagur: Ng and Kottelat, 2008, *Zoological Journal of the Linnean Society*, 153: 725-732 (status discussed).

Local Name: Magur

Common Name: Wagur, Mangur, Manguri

Materials Examined: 10 ex., 79.5- 465 mm SL. India: Uttar Pradesh: Ganga Channel at the border of Unnao & Raibareli Districts, Ganga river drainage, Pratima Singh, 7 March 2011.

Diagnosis: A species of *Clarias* with anterior margin of the pectoral spine with distinct serrations, wider snout with rounded lateral margins in dorsal view, axillary barbelalomost twice as long as the head, and chromosome number (2*n*) of 50-54 and an arm number (FN) of 58-88.

Distribution: Clariasmagur is distributed in Ganga and Brahmaputra river basins in northern and northeastern India, Nepal, Bhutan and Bangladesh (Ng and Kottelat 2008). In India it is reported from Arunachal Pradesh, Assam, Bihar, Chandigarh, Chattisgarh, Darjiling, Delhi, Haryana, Himachal Pradesh, Jharkhand, Madhya Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Tripura, Uttaranchal, Uttar Pradesh, West Bengal (Vishwanath, 2010).

Remarks: Clariasmagur was described by Hamilton (1822) from Gangetic provinces. The identity of the southern form of Clarias requires verification. The range of distribution of Clariasbatrachus is now restricted to Sunda Islands (type locality Java) (Ng and Kottelat 2008). It breeds in shallow marginal waters of ponds, ditches, and inundated paddy-fields during summer monsoon and rainy season. Currently, IUCN (2018) assessed the species as endangered.

In India and Bangladesh, it has a fish of high demand and attracts the attention of farmers for its high market value. This species is very much prevalent in Bangladesh due to use as an important part of the diet for children and lactating mothers and also prescribed as a diet for the convalescent of the patients. Furthermore, the species can be kept alive for a long time by storing in a water container without giving any food as the species bear special accessory respiratory organ. This fish is highly regarded for food due to its high protein, low fat and high iron content (Vishwanath, 2010).

Morphology of Gonads

The ovary of Clariasmagur is a paired elongated sac-like structure lying ventrally on either side of the air bladder and is attached to the coelom by a thin mesovarium which appears like strands. The ovary of the left half is generally, slightly larger in length than the right, but in several specimens, both are found equal in length. They remain separated from one another throughout the length except for the caudal or posterior ends which fuse to form a common oviduct. The oviduct posteriorly is prominent in immature fishes but indistinguishable in mature species. The common oviduct unites posteriorly with the common ureter to form urogenital sinus which terminates at the urogenital papillae. The urogenital papillae are a short and broad structure having a longitudinal slit-like furrow near the opening.

On the other hand, the male has an elongated, narrow muscular sac-like structure. Thus the *C. magur* shows sexual dimorphism particular during the breeding season. The female looks slightly heavier with short, oval slit like papillae and the male is slender having elongated conical papilla. The outer surface of the ovary is smooth during the non-breeding period but becomes rough at mature due to the bulging of ripe ova, which can be seen through the transparent ovarian wall. The length, width and the color of the ovary change according to the stages of maturity as it is creamy pinkish during immature or resting phase but becomes reddish- brown with a high degree of vascularization during maturity or pre-spawning and spawning stages.

Ovarian Cycle

From general appearance, duration of different stages of the developing oocytes and the histological peculiarities of the ovary, the ovarian cycle in Clariasmagur may be divided into following phases.

Phase I - Resting Phase (November - December)

The ovaries are dirty white in color, translucent with inconspicuous vascularisation. The vascularization is mostly confined to the ovarian well region. The ovaries occupy a minimal area of the body cavity. It shows ovarian wall with ovigerous lamellae or folds having clusters of oogonia and numerous immature oocytes.

Phase-II- Preparatory or Early Maturing Phase (January - February)

The color of the ovary becomes pale yellowish. Volume and the size gradually increased and occupied about half of the body cavity. The vascular supply is still inconspicuous. This phase corresponds to the immature and early maturing oocytes.

Phase - III - Pre Spawning Phase (May - June)

During this phase, the ovary becomes reddish brown in color due to the presence of a considerable number of mature eggs. The bulging of ova gradually becomes more and prominent on the external surface of the ovarian wall. The ovary attains about three-fourth space of the body cavity. During this period, the yolk deposition in ova is most extensive. Atretic follicles of different stages eggs are also observed, which are placed closely and obliterates the entire ovarian lumen.

Phase - IV- Spawning Phase (July - August / September)

During this phase, the ovary attains their maximum weight and width. The color becomes reddish brown; deeper than the pre-spawning stage. The ovarian surface is maximumly bulged due to the bulgings of ova. The ovary occupies a large area of the body cavity. The blood supply also increases enormously, and this phase includes all the stages of developing oocytes, atretic follicles, and yolk formation. A large number of ripe eggs are ready for spawning.

Phase V - Spent Phase or Post-Spawning Phase (Sep - October)

This phase starts just after the spawning of fish or spawning phase. During this phase, the ovary becomes very much shrunken, highly vascularised and reddish in color. The length width and weight of the ovary become reduced. The ovarian wall becomes comparatively thick and vascularised. Discharged and degenerating follicles and immature oocytes including oogonia very common. Many interfollicular spaces are also visible.

Histology of Gonads

The ovary is a typical teleostean type consisting of an outermost peritoneal membrane below which includes of the ovarian wall and the developing ova. Apart from the peritoneal, the ovarian wall is composed of two layers. The outer tunica albuginea and inner-germinal epithelium. The tunica albuginea consists of connective tissue, muscle fibers, and blood capillaries. It is thickened during the non-breeding season. The germinal epithelium consists of a single layer of cuboidal cells containing scanty cytoplasm and relatively large sized deeply stained spherical nucleus having nucleoli. At many places, the germinal epithelium loses its contact with the inner surface of tunica albuginea and projects into the lumen (ovarian cavity) to form ovigerous lamellae. Each lamella is formed of connective tissues, blood capillaries, and germinal epithelium. In young ovaries, these lamellae are thin, but in mature condition, they are swollen due to the presence of a large number of enlarged ova, which almost wholly fitted up the ovarian cavity. These ovigerous lamellae are the seat of the development of a crop of oogonia.

Egg Membrane

The early oocytes or oogonia are surrounded by a thin non-cellular layer, perhaps formed by the hardening of the outer layer of the cytoplasm. This layer is called vitelline membrane or zone radiata. The new oocytes are either produced by oogonial nests or by the residual oocytes after passing spawning period all oocytes do not ripe simultaneously throughout the ovary, hence before an ovum ripes each oocyte passes through a series a maturation stages during this process, growing oocytes become enveloped by a follicular epithelium, which becomes distinct in mature oocytes, where it has well defined an outer layer made up of theca cells and inner of granulosa cells, below which is the zone radiata. In some teleosts, the theca layer is further divided into theca externa and theca interna, but not so found in present fish, Clariasmagur. The theca layer becomes distinct only in ripe or fully matured oocytes in which zonaradiata become striated, which might be pore canals.

Oocytes: On the basis of yolk nuclear changes and yolk deposition, the developing oocytes of Clariasmagur may be divided into following stages.

Oogonium (Stage - I)

The oogonium is the earliest stage of eggs formed from the germ cells of the ovary and generally in clusters in ovigerous folds. It is characterized by its small size, presence of a large strongly basophilic nucleus, very small size, the presence of a large strongly basophilic nucleus, very small amount of basophilic cytoplasm and complete absence of yolk. The amount of the cytoplasm gradually increases.

Immature Oocyte (Stage-II)

The oogonium becomes larger due to increase in the cytoplasm and surrounds the centrally placed nucleus, which now contains 2-4 strongly basophilic nucleoli scattered in the nucleoplasm. The cytoplasm remains basophilic in nature. In the later part of the development, there is a numerical increase in the number of nucleoli, which arranged themselves at the periphery of the nucleus, beneath the nuclear membrane. A yolk nucleus just close to the nuclear membrane is observed, which gradually moves towards the periphery of the cytoplasm with the distorted condition in growing oocytes. No yolk is yet formed.

Maturing Oocytes (Stage - III)

The oocytes become oval in shape, and the size also increased the yolk nucleus which becomes fragmented into pieces at the periphery of the ooplasm and gradually disintegrates. This stage is characterized by the little formation of yolk, as the yolk vesicles appear in the peripheral zone of the ooplasm. Gradually these vesicles increase in number and size centripetally as the oocytes advance with maturity and finally the whole ooplasm are fitted up with these yolk vesicles. Some of the yolk vesicles seen to be empty whereas many have yolk bodies. The number of nucleoli the nucleus increases by division or fragmentation and themselves on the periphery just beneath the nuclear membrane. Gradually the nuclear membranes become amoeboid appearance as many out pocket (Pseudopodial outgrowths) are formed consisting nucleoli. Gradually, these nucleoli phased out intact into the ooplasm, where it becomes disintegrated and moved up with the ooplasm. At this stage, yolk globules or inter vesicular yolk appears in the ooplasm and in between the yolk vesicles and gradually their size and number increase with the development of oocytes.

In the later part of the growth, the nucleus gradually moves towards the periphery of the oocytes of the ooplasm and cortical alveoli. The follicular epithelium forms distinct separate layers around the oocyte. Zone radiata which is also basophilic grows in thickness. The yolk vesicles are positive to PAS Sudan black 'B' and oil red 'O' but negative to 'AB' (pH 1.0 - 3.0) indicating the presence of sulfated mucopolysaccharide and lipid materials.

Mature Oocytes (Stage - IV)

These are characterized by almost disappearance of the nuclear membrane and heavy deposition of the yolk of both types in yolk vesicles and yolk globules (or intravascular yolk). Gradually, with the development or when the egg becomes fully ripe, yolk vesicles steadily reduced in size and number whereas yolk globules increase their size and form a homologous continuous mass of yolk. There are distinct layers of follicular epithelium (outer theca and inner zone granulosa). Among the two layers, zone granulosa is syncytial in nature having deeply stained nuclei, takes an active part in the formation of atretic follicles (atresia). Some striations are visible in zone radiate which might be the pore canals.

Yolk Formation

The yolk nucleus first appears close to the nuclear membrane, then gradually moves towards the periphery, becomes fragmented and finally disappears. This coincides with the formation of yolk vesicles in the developing oocytes. Similarly, other types of yolk globules or inter vesicular yolk appears first around the nuclear extrusion as the extruded nucleoli, disintegrate and mixed up with the ooplasm. Later, the yolk globules increase in size and number and become scattered in the whole of the ooplasm and in between the yolk vesicles. Thus the presence of two types of the yolk is fully marked in the oocytes of IIIrd and IVth stages of development.

Atretic Oocytes and Its Formation

Many oocytes, which undergo resorption, are known as atretic oocytes, and the process is called follicular atresia. Degeneration of oocyte is the most common feature during maturation, i. e., pre-spawning, spawning and immediately after spawning (i. e., post-spawning) periods, but it was not found in resting or preparatory period or in immature oocyte stage.

Theca and granulosa cells take an active part in the formation of atretic follicles, as their number increase and loose regular appearance. At first granulosa cells and then theca cells enter into the cytoplasm along with blood capillaries or inter ovular spaces, by breaking the oolemma and start phagocytosis of the ovum (ooplasmic contents). The nuclear apparatus disappears in such oocytes.

The following stages of atretic follicles are observed in the ovary of Clariasmagur during its gonadal cycle.

Stage – I

The theca cells do not show any apparent change, but the granulosa cells begin to hypertrophy. It loses its syncytial nature and becomes cellular in an appearance. The zone radiate is deflected off from the granulosa layer to form finger-like projections. Nucleus becomes disappeared. Small yolk globules fuse together to create bigger spherical masses.

Stage – II

Further hypertrophy of the granulosa layer is evident. Zonaradiata becomes ruptured, and through it, cytoplasmic granules of follicle cells invade the yolk. The liquefaction of yolk starts towards the peripheral zone of the oocytes.

Stage III

The yolk liquified as active phagocytosis digests them. The theca cells a: The zone radiata becomes completely disintegrated and present, in traces. Most of nd blood cells penetrate into the ooplasm, which shows vascularisation. Most of the yolk finally is replaced by granulosa cells.

Stage – IV

The entire mass of the atretic oocytes becomes more compact with a yellowish patch of yellow pigments. The follicular cells become reduced in one or two layers of flattened cells and indistinguishable from theca and granulosa layers.

Stage – V

The discharged follicles, i. e., after the discharge of the eggs, the theca and granulosa cells are left behind, and the space or lumen of the follicle gradually filled up by the proliferation of zona granulosa and theca cells. These stages are called post-ovulatory corpus luteum and are common in the ovary, just after spawning period ovulatory phases. Finally, these follicles are reabsorbed by ovarian tissue.

The gonadosomatic index (GSI) ranged from 0.27 during December to 12.05 during August. The GSI value was higher during the monsoon season, i. e., from June to September with peak value observed during August. The average ovary weight ranged from 0.175 g during November to 8.520 g during August. Similarly, average ova diameters were ranged from 107 µm in the month of December to 964µm in the month of August. The seasonal variations in the average weight of the ovary along with ova diameter and G. S. I. suggests that the spawning period of this fish is from the last part of June to first part of September. The fish attains its maturity for spawning after the commencement of good rain. Further, the ova diameter measurement also indicates that in a single season, a single large group of oocytes usually shed, which shows a definite spawning period in a year.

C. magur exhibits sexual dimorphism. The sexes can be identified by a careful examination of the urogenital openings and related papillae, as the males have an elongated genital papilla whereas, the females have rounded and comparatively short. The ovary of Clariasmagur is of "Cystovarian" type as the lumen of the ovary is continuous with small oviduct posteriorly; while on the basis the arrangement of follicles, it corresponds to the "Cyprinodont type" under the classification Brock (1878). The ovary consists of the ovarian wall, oogonia, oocytes surrounded by follicular epithelium, stroma or surrounding tissue and vascular and nervous tissues. Its color and size vary at different times in the gonadal cycle.

Several workers studied the yolk nucleus in many fishes, but there is no agreement on them regarding the origin and function of this structure (Narain, 1937, Chopra, 1961, Nayyar, 1964, Wheeler, 1924, Chaudhary, 1952, Bara, 1960). In Clariasmagur, yolk nucleus makes its appearance just near the nuclear membrane in the ooplasm during immature oocyte stage. It is strongly basophilic and rich with RNA.

CONCLUSIONS

In most of the poikilothermic animals, the reproductive activity is rhythmic, and breeding phase becomes restricted to a particular season of the year. Many works have reported on spawning periodicities in different fishes (Clark, 1934; De Jong, 1939; Sathyaneshan, 1961). Considering the nature and presence of oocytes, examining the ova-diameter,

gonadosomatic index and histological structure of mature oocytes, it appears that C. magur, spawn only once in a year as single large group of riped oocytes would shed in a single season, indicating a definite spawning period from July (or late June) to August half of September, perhaps depending on the rainfall. Therefore, it may be placed under the "group synchronism."

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